Reply to Notice of Non-Compliant Amendment dated May 12, 2009

Amendment dated June 1, 2009

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the

application:

1. (Currently Amended) A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding entity

and a first oligonucleotide comprising a first portion wherein the first analyte-specific binding

entity is capable of forming a complex with the analyte and is conjugated to the first

oligonucleotide;

(c) a second proximity member, comprising a second analyte-specific binding

entity and a second oligonucleotide comprising a portion that is capable of hybridizing to the

first portion of the first oligonucleotide wherein the second analyte-specific binding entity is

capable of forming a complex with the analyte and is conjugated to the second oligonucleotide

comprising a portion that is capable of hybridizing to the first portion of the first oligonucleotide;

and

(d) a hybridization blocker oligonucleotide at a concentration in excess of the

concentration of the first and second proximity members, wherein the hybridization blocker

oligonucleotide comprises a portion that is capable of forming a hybrid with the first portion of

the first oligonucleotide to reduce hybridization between the first and second oligonucleotides

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and wherein the first and/or second analyte-specific binding entity is a protein;

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(ii) forming a complex comprising the analyte, the first proximity member, and the

second proximity member, and the hybridization blocker oligonucleotide that is hybridized with

the first portion of the first oligonucleotide;

(iii) forming a hybrid by displacing the hybridization blocker oligonucleotide wherein

the hybrid comprises the first portion of the first oligonucleotide and the portion of the second

oligonucleotide, wherein the hybrid comprises a 3' terminus of the first or second oligonucleotide

that may be extended;

(iv) extending the 3' terminus of the first or second oligonucleotide and producing an

amplicon;

(v) amplifying the amplicon and producing an amplification product; and

(vi) detecting the amplification product, wherein detection of the amplification

product allows indicates detection of the analyte.

2. (Previously Presented) The method of claim 1, wherein the hybridization blocker

oligonucleotide comprises a 3' sequence that is not complementary to the first oligonucleotide.

3. (Previously Presented) The method of claim 1, wherein the hybridization blocker

oligonucleotide comprises a 5' sequence that is not complementary to the first oligonucleotide.

4. (Previously Presented) The method of claim 1, wherein the hybridization blocker

oligonucleotide comprises a 3' sequence and a 5' sequence that are not complementary to the first

oligonucleotide.

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 (Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide comprises a 3' cap that prevents 3' extension of the hybridization blocker oligonucleotide by a DNA polymerase.

- 6. (Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid with the first portion of the first oligonucleotide wherein the hybridization blocker oligonucleotide contains bases that form a hybrid-are complementary with all of the bases of the first portion of the first oligonucleotide comprising all of the bases of the first portion of the first-portion of the first-portion of the first-portion.
- (Previously Presented) The method of claim 6, wherein the first portion of the first oligonucleotide is about 10 bases in length and the hybridization blocker oligonucleotide is about 18 bases in length.
- 8. (Currently Amended) The method of claim 6, wherein the length of the first portion of the first oligonucleotide is about the length of the [[entire]] first oligonucleotide.
- 9. (Withdrawn-Currently Amended) The method of claim 1, wherein the hybridization blocker oligonucleotide forms a hybrid with the first portion of the first oligonucleotide wherein the bases of the hybridization blocker oligonucleotide of the hybrid are emprising less than all of the bases of the first portion of the first oligonucleotide.

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(Currently Amended) The method of claim 1, further eomprising adding
 combining a deblocker oligonucleotide that is capable of reducing the hybridization between the

hybridization blocker oligonucleotide and the first oligonucleotide.

11. (Previously Presented) The method of claim 10, wherein the deblocker

oligonucleotide comprises a first portion that is capable of forming a hybrid with the portion of

the hybridization blocker oligonucleotide that is capable of forming a hybrid with the first

portion of the first oligonucleotide.

12. (Previously Presented) The method of claim 11, wherein the deblocker

oligonucleotide comprises a second portion that is capable of forming a hybrid with the portion

of the hybridization blocker oligonucleotide that does not form a hybrid with the first portion of

the first oligonucleotide.

13. (Currently Amended) The method of claim 1, wherein the hybridization blocker

oligonucleotide comprises a double-stranded portion. [[that]] wherein the double-stranded portion

is 3' of the portion of the hybridization blocker oligonucleotide that is capable of forming a

hybrid with the first portion of the first oligonucleotide.

14. (Original) The method of claim 13, wherein the double-stranded portion

comprises a hairpin loop.

(Cancelled)

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16. (Withdrawn) The method of claim 10, wherein the hybridization blocker

oligonucleotide hybridizes with the first portion of the first oligonucleotide after the addition of

the deblocker oligonucleotide.

17. (Withdrawn-Currently Amended) The method of claim 10, wherein the

hybridization blocker oligonucleotide hybridizes is added simultaneously with the deblocker

oligonucleotide.

18. (Withdrawn) The method of claim 1, wherein the hybridization blocker

oligonucleotide is added before the combination of the analyte and first and second proximity

members.

(Withdrawn) The method of claim 1, wherein the hybridization blocker

oligonucleotide is added after the combination of the analyte and first and second proximity

members.

(Currently Amended) The method of claim 1, further comprising adding in step

(i) a second hybridization blocker oligonucleotide at a concentration in excess of the

 $\underline{concentration\ of\ the\ first\ and\ second\ proximity\ members}, \ [[that]]\underline{wherein\ the\ second}$

hybridization blocker oligonucleotide is capable of hybridizing to the portion of the second

oligonucleotide wherein the portion of the second oligonucleotide is capable of forming a hybrid

with the first portion of the first oligonucleotide, forming a complex in step (ii) further

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comprising the second hybridization blocker oligonucleotide hybridized to the portion of the second oligonucleotide, and displacing in step (iv) the second hybridization blocker

oligonucleotide.

21. (Previously Presented) The method of claim 1, wherein said amplifying the

amplicon is by a method selected from the group consisting of polymerase chain reaction, strand

displacement amplification, thermophilic strand displacement amplification, self-sustained

sequence replication, nucleic acid sequence-based amplification, QB replicase system based

amplification, ligase chain reaction, and transcription mediated amplification.

22. (Currently Amended) The method of claim 1, wherein the hybridization blocker

oligonucleotide reduces formation of the amplicon by hybridization of the first and second

oligonucleotides prior to forming [[a]]said complex by a factor of at least 100-fold as detected in

assay relative to amplicon formation without a hybridization blocker oligonucleotide.

23. (Currently Amended) The method of claim 22, wherein the hybridization blocker

oligonucleotide reduces formation of the amplicon by a factor of at least 1000-fold as detected in

assay relative to amplicon formation without a hybridization blocker oligonucleotide.

24. (Currently Amended) The method of claim 1, wherein the analyte is capable of

being detected when the concentration of the analyte is at least about 1 pM.

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25. (Previously Presented) The method of claim 1, wherein the analyte is capable of

being detected when the concentration of the analyte is at least about 0.1 pM.

26. (Previously Presented) The method of claim 1, wherein the analyte is capable of

being detected when the concentration of the analyte is at least about 0.01 pM.

27. (Previously Presented) The method of claim 1, wherein the detecting the

amplification product is quantitative.

28. (Previously Presented) The method of claim 1, wherein the first or second

analyte-specific binding entity is a protein complex.

29. (Previously Presented) The method of claim 28, wherein the protein complex

comprises a first protein that is conjugated to the first or second oligonucleotide and a second

protein that is capable of forming a complex with the analyte.

30. (Original) The method of claim 29, wherein the first protein is selected from the

group consisting of Protein A and Protein G.

31. - 73. (Cancelled)

74. (Currently Amended) A method of detecting an analyte, comprising:

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(i) combining:

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(a) an analyte;

(b) a first proximity member, comprising a first analyte-specific binding

entity and a first oligonucleotide comprising a first portion and a second portion

comprising a 5' terminus wherein the first analyte-specific binding entity is

capable of forming a complex with the analyte and is conjugated to the first

oligonucleotide:

(c) a second proximity member, comprising a second analyte-specific

binding entity and a second oligonucleotide comprising a first portion wherein the

second analyte-specific binding entity is capable of forming a complex with the

analyte and is conjugated to the second oligonucleotide, wherein the first portion

of the first oligonucleotide is capable of hybridizing to the first portion of the

second oligonucleotide and the first and/or second analyte-specific binding entity

is a protein;

(ii) forming a complex comprising the analyte, the first proximity member, and the

second proximity member, wherein the complex contains at least one hybrid comprising the first

portion of the first oligonucleotide and the first portion of the second oligonucleotide, wherein

the at least one hybrid comprises [[a]]the 3' terminus of the second oligonucleotide that is

capable of being extended to form a complement of the second portion of the first

oligonucleotide;

(iii) extending the 3' terminus and producing an amplicon;

(iv) amplifying the amplicon and producing an amplification product; and

(v) detecting the amplification product, wherein detection of the amplification

product allows indicates detection of the analyte.

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75. (Canceled)

76. (Currently Amended) The method of claim 74, further comprising

adding combining in step (i) a splint oligonucleotide comprising a first portion and a second

portion, wherein a hybrid is formed wherein [[a]]the first portion of the splint oligonucleotide

hybridizes with the first portion of the first oligonucleotide and a second portion of the splint

oligonucleotide hybridizes with the first portion of the second oligonucleotide.

77. (Canceled)

78. (Currently Amended) A method of detecting an analyte, comprising:

(i) combining:

(a) an analyte:

(b) a first proximity member, comprising a first analyte-specific binding

entity and a first oligonucleotide comprising a first portion and a second portion comprising a 5'

terminus wherein the first analyte-specific binding entity is capable of forming a complex with

the analyte and is conjugated to the first oligonucleotide;

(c) a second proximity member, comprising a second analyte-specific

binding entity and a second oligonucleotide comprising a first portion wherein the second

analyte-specific binding entity is capable of forming a complex with the analyte and is

conjugated to the second oligonucleotide, wherein the first portion of the first oligonucleotide is

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capable of hybridizing to the first portion of the second oligonucleotide and the at least first

and/or second analyte-specific binding entity is a protein;

(ii) forming a complex comprising the analyte, the first proximity member,

and the second proximity member, wherein the complex contains at least one hybrid comprising

the first portion of the first oligonucleotide and the first portion of the second oligonucleotide,

wherein the at least one hybrid comprises [[a]]the 3' terminus of the second oligonucleotide that

is capable of being extended to form a complement of the second portion of the first

oligonucleotide:

(iii) producing an amplicon comprises: (i) adding a third oligonucleotide and

forming a hybrid between the second portion of the first oligonucleotide and the third

oligonucleotide and (ii) ligating the 3' terminus of the second oligonucleotide to a 5' terminus of

the third oligonucleotide:

(iv) amplifying the amplicon and producing an amplification product; and

detecting the amplification product, wherein detection of the amplification (v)

product allows-indicates detection of the analyte.

79. - 114. (Cancelled)